

Supporting Information: 3D Printing of Regenerated Silk Fibroin and Antibody-Containing Microstructures via Multi-Photon Lithography

Matthew B. Dickerson¹, Patrick B. Dennis¹, Vincent P. Tondiglia¹, Lloyd J. Nadeau¹, Kristi M. Singh¹, Lawrence F. Drummy¹, Benjamin P. Partlow², Dean P. Brown¹, Fiorenzo G. Omenetto², David L. Kaplan², and Rajesh R. Naik^{3*}

1. Materials and Manufacturing Directorate, Air Force Research Laboratory, Wright-Patterson AFB, OH

2. Biomedical Engineering Department, Tufts University, Medford, MA

3. Human Performance Directorate, Air Force Research Laboratory, Wright-Patterson AFB, OH

*Correspondence: Rajesh.Naik@us.af.mil

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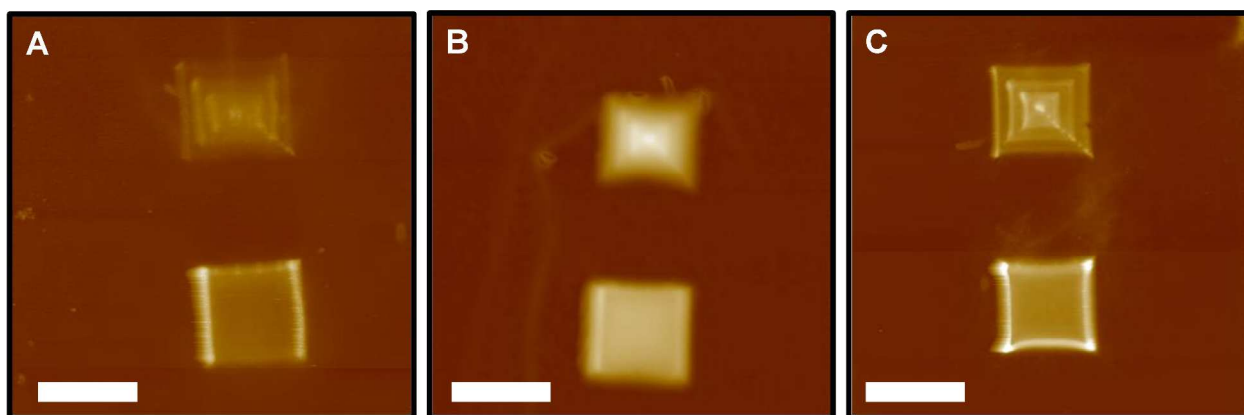


Figure S1: AFM characterization of test structures (top- pyramid and bottom- block) produced via the multi-photon polymerization of RSF solutions utilizing different photosensitizers to initiate protein crosslinking. The photosensitizers used were A) methylene blue (0.75 mM), B) $\text{Ru}(\text{bpy})_3^{2+}$ (10 mM), and C) rose bengal (10 mM). All scale bars represent 10 μm . The Z-axis is 2 μm .

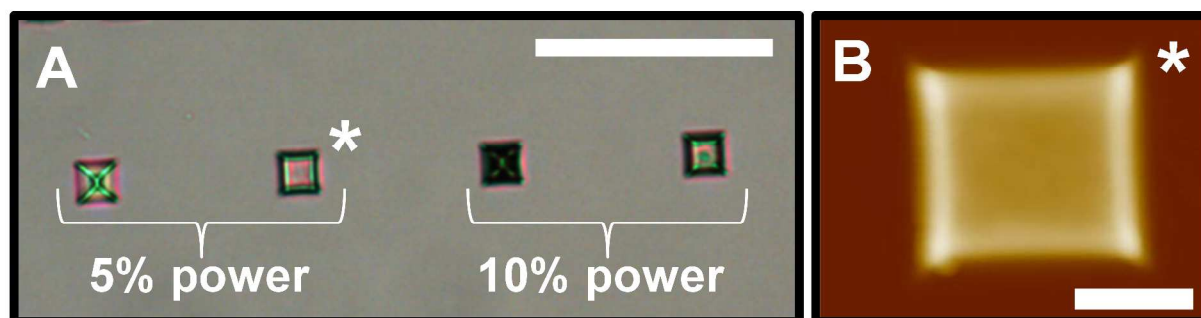


Figure S2: MPL production of RSF structures utilizing $\text{Ru}(\text{bpy})_3^{2+}$ with ammonium persulfate (APS). A) Optical microscope images of sets of structures (step pyramid (left) and block (right)) produced at laser powers (5% and 10%) lower than that used to build similar structures without using APS (typically 50% laser power). AFM characterization of a block test structure produced at 5% laser power (same structure as highlighted with an asterisk in A). Scale bars represent 50 and 5 μm in A) and B), respectively. The Z-axis in B) is 3 μm .

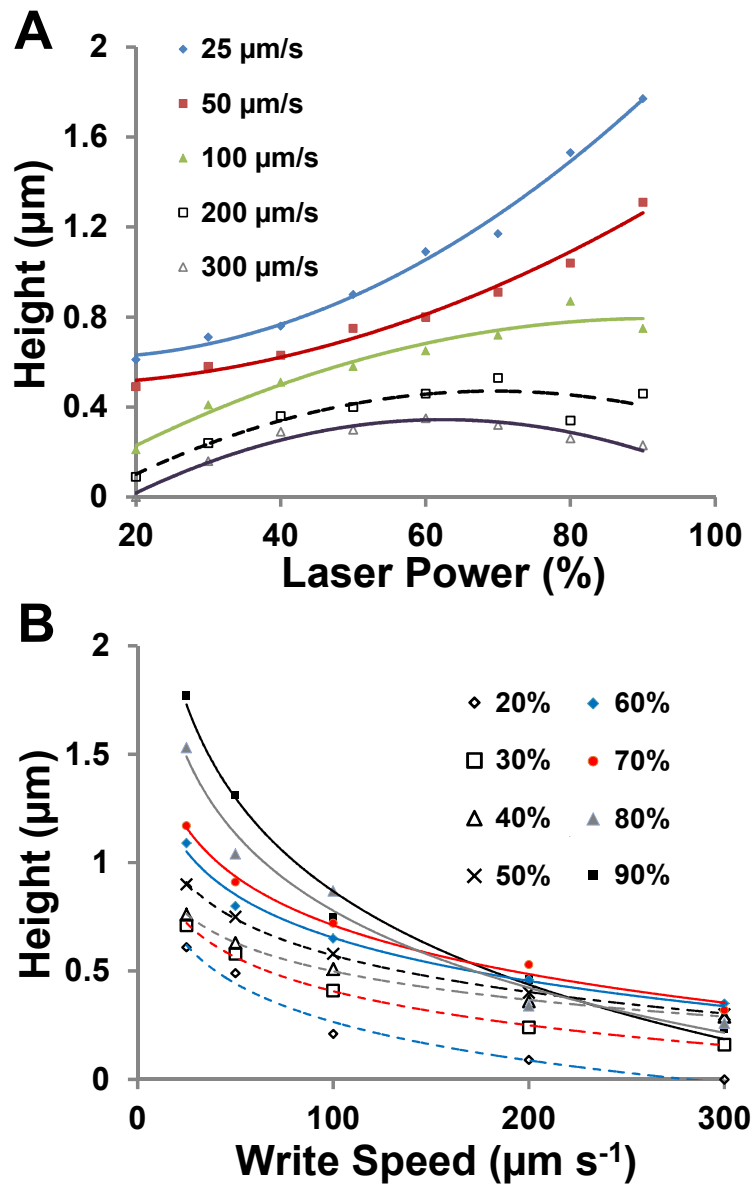


Figure S3: Plots of the height of RSF block structures as a factor of processing conditions. A) Dependence of block height (2 μm design height) as a function of laser power at various writing speeds. B) Dependence of block height as a function of writing speed at various laser power settings. Identical data presented in both plots.

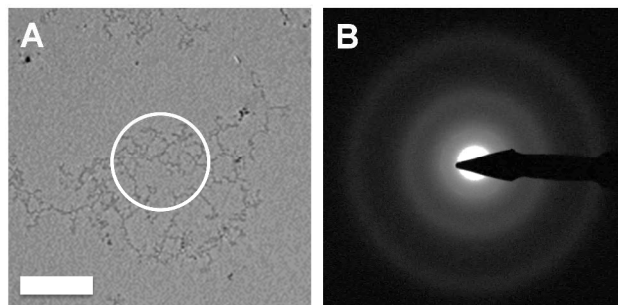


Figure S4: TEM characterization of MPL-polymerized RSF structures. A) TEM brightfield imaging of a microtomed RSF structure cross-section. Scale bar represents 5 μm . B) Selected area electron diffraction of RSF circled in A. Diffraction rings correspond to the 0.45 nm silk II β -sheet (200)/(210) reflections.

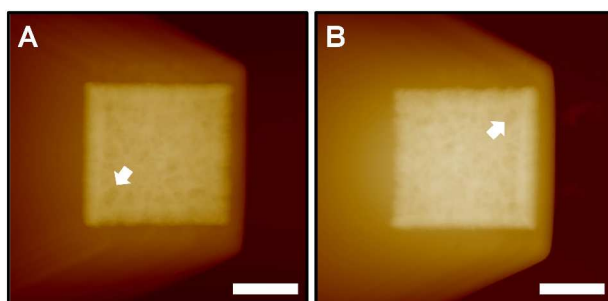


Figure S5: Demonstration of imaging artifact observed during AFM characterization of test RSF/IgG structures (block) while submerged in water. The AFM tip traveled in the left-right direction during imaging. The identical RSF/IgG sample is depicted in both A and B, but physically rotated 180° between the scans. A distinctive morphological feature (a pore) is designated by the white arrows. The wake-like structure is observed to follow the AFM probe scanning direction rather than the physical sample. This indicates that the “wake-like” or “fantail” characteristic present in the images is an artifact. All scale bars represent 10 μm and the Z-axis is 20 μm in both images.

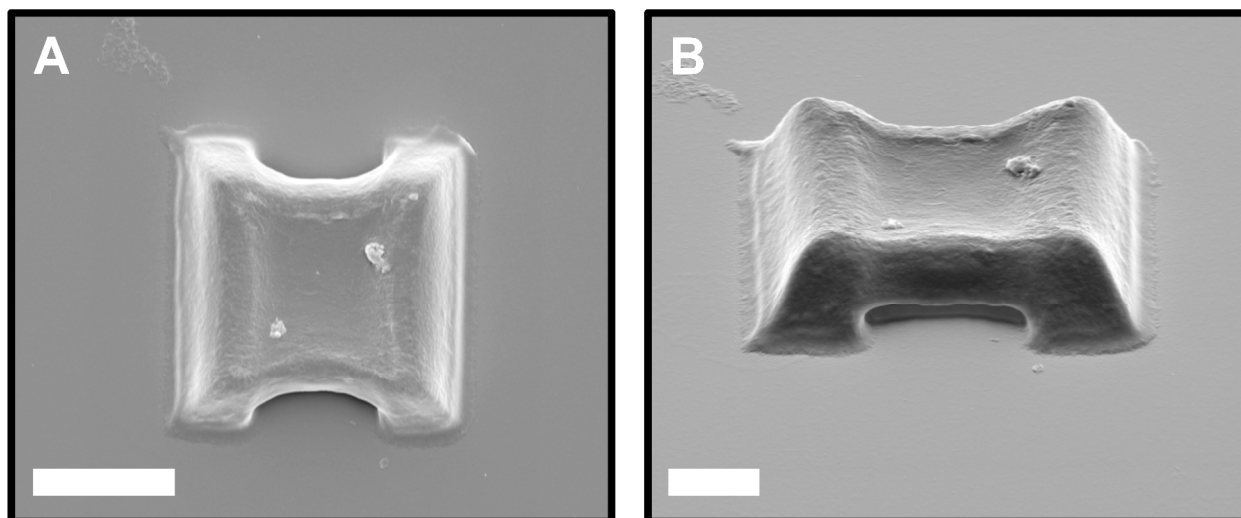


Figure S6: SEM images of a RSF/ $\text{Ru}(\text{bpy})_3^{2+}$ arch printed by MPL. A) Top-down view and B) side view of RSF arch. Though some subsidence is visible in B), the spanning structure appears to be self-supporting. Scale bars represent 5 μm .

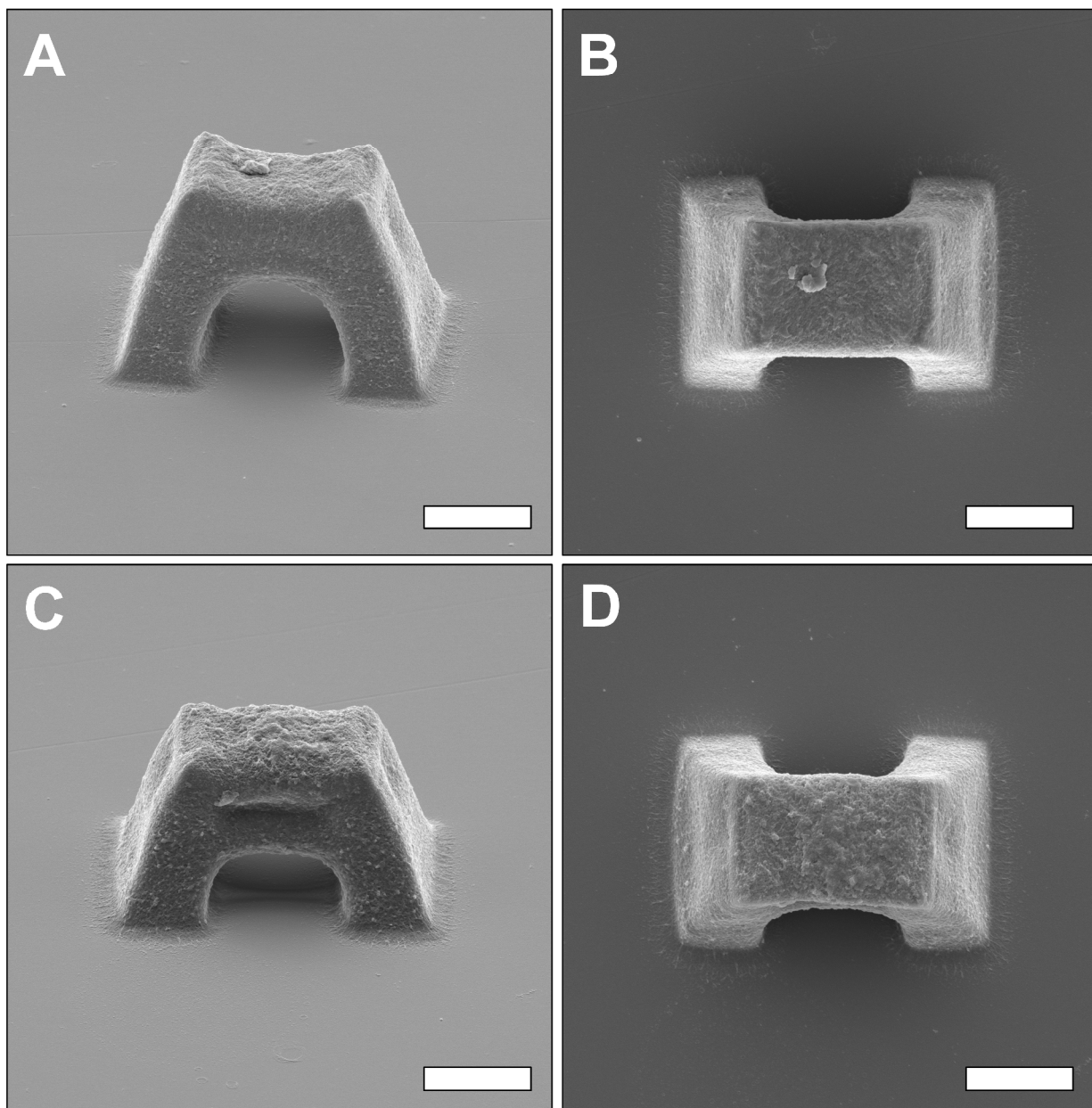


Figure S7: SEM images of a RSF/ $\text{Ru}(\text{bpy})_3^{2+}$ free-spanning arches printed by MPL and subjected to additional crosslinking conditions (MPL-3P) to increase the rigidity of the structure. A) Side view and corresponding B) top-down view of a RSF arch. Another example of a self-supporting RSF structure is depicted in C) side view and D) corresponding top-down view. Scale bars represent 5 μm .